SUPPLEMENTARY METHODS

Study population

From March 2019 to October 2020, we enrolled 56 patients on chronic hemodialysis (HD) and 18 healthy controls at Avantus Irving Place Dialysis Center, Upper East Dialysis Center, and Mount Sinai Hospital (all in New York, NY).

All the patients were treated with conventional HD three times a week as per their regular schedule, and they have been on HD for at least 3 months before the enrollment.

As we evaluated arterial oxygen saturation (SaO_2), we included in the study only patients with arteriovenous access (arteriovenous fistula, AVF). Previous studies demonstrated a strong correlation in blood gases levels in samples obtained from the radial artery and from a well-functioning AVF,[S3] therefore our measurements accurately reflected SaO_2.

Other exclusion criteria were the presence of signs of infection, malignancies, severe anemia (Hb < 7 g/dl), and genetic red blood cells (RBC) disorders known in the patients' clinical history.

All patients and controls provided written informed consent to study participation.

Prolonged intradialytic hypoxemia (PIH)

Prolonged intradialytic hypoxemia (PIH) was defined as arterial oxygen saturation below 90% for at least one third of the treatment time and was assessed considering the treatments performed over the three months prior enrolment.

Intradialytic SaO_2, the parameter used to estimate intradialytic hypoxemia, was measured by the Crit-Line Monitor (CLM, Fresenius Medical Care North America, Waltham, MA), an

FDA-approved device that provides non-invasive measurement of oxygen saturation levels every minute.

Eryptosis, reactive oxygen species (ROS), and reticulocytes measurement

Blood samples were collected before dialysis session and analyzed within 2 hours to prevent alteration in intracellular reactive species (iROS) levels.

To evaluate RBC apoptosis (eryptosis), we stained the cells with annexin-V (FITC; Becton Dickinson, San Jose, CA, USA) and analyzed them by flow cytometry [three-laser FACSLyric flow cytometer (BD Biosciences)].

We measure iROS in RBCs by flow cytometry with ROS-ID® Total ROS/Superoxide detection kit (Enzo Life Sciences, Farmingdale, NY, USA), following protocol instructions.

The percentages of reticulocytes were assessed using Retic-COUNT (thiazole orange, TO; Becton Dickinson, San Jose, CA, USA). The cells were co-stained with anti-CD71 antibody (transferrin receptor; APC, Becton Dickinson, San Jose, CA, USA). As previously published, based on this marker expression, we identified three subsets of reticulocytes: immature ($TO^{LOW}CD71^{HIGH}$), intermediate ($TO^{LOW}CD71^{INT}$), and mature ($TO^{LOW}CD71^{LOW}$). TO^{HIGH} cells identified small lymphocytes and were excluded from analyses.

Statistical analyses

Two group comparisons were analyzed by Mann-Whitney or Chi-squared test. *P* values < 0.05 were considered significant. All statistical analyses were performed using GraphPad Prism (version 8 for Windows, GraphPad Software, Inc.).

SUPPLEMENTARY REFERENCES

S1. Meyring-Wösten A, Kuntsevich V, Campos I*, et al.* Erythrocyte Sodium Sensitivity and Eryptosis in Chronic Hemodialysis Patients. *Kidney and Blood Pressure Research* 2017; **42:** 314-326

S2. Tozoni SS, Dias GF, Bohnen G, *et al*. Uremia and Hypoxia Independently Induce Eryptosis and Erythrocyte Redox Imbalance. Cell Physiol Biochem. 2019;53:794-804.

S3. Nielsen AL, Thunedborg P, Brinkenfeldt H*, et al.* Assessment of pH and oxygen status during hemodialysis using the arterial blood line in patients with an arteriovenous fistula. *Blood Purif* 1999; **17:** 206-212.

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